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Factors influencing the bacteriological quality of sausages sold in Meknes city, Morocco

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Abstract

The aim of this study was to determine the bacteriological quality of sausages made from beef meat, turkey meat and artisanal sausages (undetermined origin) sold in Meknes city, Morocco in different sites: street vendors, butchery, Supermarket and Souk (Weekly market). For this reason, 156 samples were taken over a year, from March 2014 until in February 2015. The samples were submitted to enumeration of Total Aerobic Bacteria (TAB), Total coliforms (TC), Faecal coliforms (FC), *Escherichia coli (E. coli), Staphylococci* (St), *Clostridium perfringens* (Cp) and *Salmonella.* The results of this study show that only 19.23% (30/156) of samples meet the standards hygiene, with an average contamination of: 7.13 for TAB; 5.05 for TC; 4.06 for FC; 3.69 for *E. coli*; 3.43 for St and 2.42 for Cp in log CFU/g, *Salmonella* was detected in 21.79% of samples. However, the contamination level depends on sausage type, sampling site, and the seasonal variation intimately related to the change in temperature.

Introduction

The term sausage is derived from the Latin word "salsus" meaning salt, or, literally translated, refers to chopped or minced meat preserved by salting (Pearson and Gillett, 1996). Sausages are one of the oldest forms of processed foods, their origin being lost in antiquity, it has been reported that sausages were used by the Babylonians and the Chinese about 1500 B.C. (Pearson and Gillett, 1996). It's a very popular tasty food worldwide (Mazahreh *et al*., 2013), a popular processed meat product, traditionally consists of chopped meat, water, binders, and seasonings (Essien, 2003; Louis-Sylvestre *et al.*, 2010) stuffed into natural or artificial casings prior to cooking with or without smoke application. Natural casings are made from sheep, hog, and beef intestine (Harper *et al*., 2012), while artificial casings commonly consist of collagen, cellulose, or plastic (Feiner, 2006).

The collective food-borne diseases, in humans, caused by pathogenic bacteria and their toxins are well known worldwide (Hazariwala *et al.*, 2002). They are defined by the occurrence of two cases minimum of a digestive symptoms, whose the reason can bring to the same food-borne (Delmas *et al.*, 2010), dominated mainly by diarrhea (Avignon *et al*., 2001). The food-borne diseases imposes a substantial economic burden and threatens the public health on society causing an acute morbidity and chronic sequelae (Duff *et al*., 2003; Gruber *et al*., 2015). Worldwide, 2.1 million adults and 3 million children die due to consumption water and contaminated food (WHO, 2007). It has been estimated that there are 9.4 million cases of food-borne diseases each year in the United States; of which 55 961 cases are hospitalized and 1351 deaths (Scallan *et al*., 2011). In Montreal (Canada), Between 1 April 2011 and 31 March 2012, a total of 1114 food poisoning episodes related to 1207 statements (including 39 events of allergies) were reported to the Minister of Agriculture, Fisheries and Food, These reports aimed 2859 people sick (Ramsay, 2012). In 2011, 1 153 collective food-borne diseases have been reported in France, affecting 9 674 people, including 668 hospitalized and 7 died (Erouar and Vasiliu, 2013). In Morocco, the food-borne diseases are very common and affect all regions, predominate in summer and spring. They are accidental touching adult young are usually benign although sometimes fatal (Aoued *et al*., 2010). From 1989 to 2008, the Moroccan anti poison center has collected 17 896 cases of food-borne diseases, representing 22.1% of all poisonings cases collected during the same period with 59 deaths (10 cases in Meknes-Tafilalet region) (Aoued *et al.*, 2010). Between 2001 and 2006, 630

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collective food-borne diseases were reported whose the origin of meat has been often disputed (Belomaria *et al*., 2007).

Sausage is a product, outcome of artisanal processing meat, usually consumed by people vulnerable to food poisoning, without it being aware of his risk, thanks to its failing hygienic quality. In this study, we will contribute to establish a bacteriological diagnosis, to collect a maximum of data on the hygienic quality of sausage sold in Meknes city (Morocco) and to determine the various factors influencing its quality.

Materials and Methods

Samples collection

This study was focused on 156 samples of sausages represented by 60 of turkey meat, 60 of beef meat and 36 of artisanal sausages, which were collected from different shopping sites, butchery, street vendors, supermarket and Souk (Weekly market combines the population of the small villages around Meknes city). The samples were taken in three popular area, souk and supermarket; the sampling frequency is 13 samples per month (5 made of turkey meat, 5 of beef meat and 3 of artisanal sausages). The collection was carried out during one year from March 2014 until in February 2015. The collected amount is about 40 g of sausage per sample. The samples are transferred in cooler into microbiological laboratory of the Faculty of Science Meknes. 25 g of sausage was mixed with 225 mL of buffered peptone water (Oxoid), then the mixture was ground in a Masticator (Stomacher 400 Circulator, Seward) for 1 min at 260 rotation per minute (RTM), before to prepare a series of decimal dilution to carry out the enumeration of total aerobic bacteria (TAB), total coliforms (TC), faecal coliforms (FC), *Escherichia coli (E. coli)*, *Staphylococci* (St), *Clostridium perfringens* (Cp).

The bacteriological analysis

The bacteriological analysis aims to search the microorganisms whose isolation is required by bacteriological criteria, the desired parameters are the following (Table 1): the Total Aerobic Bacteria (TAB) was counted using the incorporation technique on the medium Plate Count Agar (PCA, Biokar) according to the standard (NF V08-051, 1999). The Total coliforms (TC) and Faecal coliforms (FC), are achieved by the incorporation of Violet Red Bile Lactose Agar (VRBL, Biokar), with incubation at 30°C for Total coliforms (ISO 4832, 2006) and 44°C for Faecal coliforms (NF V08-060, 2009). The enumeration of *Staphylococci* (St) is performed according to standard (NF V08-057, 2004), using the Baird Parker medium (Biokar) with incubation at 36°C ±2°C for 24 to 48 hours. The *Clostridium perfringens* (Cp) are counted using the TSC medium supplemented with D-cycloserine (Biokar) with an anaerobic incubation at 37°C ± 2°C for 24 to 48 hours (ISO 7937, 2004). The enumeration of *Escherichia coli* is carried out by the incorporation in medium Rapid *E.coli* 2 agar (Bio-Rad), with incubation at 44°C for 18 to 24 hours (NF V08-053, 2002). The research and isolation of *Salmonella* was conducted according to the AFNOR standard (NF U47-100, 2007).

Table 1. Incubation conditions for the desired bacteria.

Bacterial Germs	Culture media	Incubation
TAB	PCA	30°C for 48 hrs
TC	VRBL	30°C for 24 to 48 hrs
FC	VRBL	44°C for 24 to 48 hrs
Staphylococci	Baird Parker Agar	37°C for 48 hrs
Clostridium perfringens	TSC	37°C for 48 hrs
Escherichia coli	Rapid *E.coli* 2 Agar	44°C for 24 to 48 hrs

TAB: Total Aerobic Bacteria, TC: Total coliforms, FC: Faecal coliforms, PCA: Plate Count Agar, VRBL: Violet Red Bile Lactose Agar, TSC: Tryptone Sulfite Cycloserine Agar.

Statistical analysis of data

The results of this study were interpreted according to rules established by the Moroccan Minister of Agriculture and Health (Table 2). Data processing and statistical analysis were performed by Microsoft Office Excel (2007). The analysis of means was performed using student test with α=0.05.

Results and Discussion

Bacteriological analysis of samples

The bacteriological analysis of sausages is shown in Table 2.

Total aerobic bacteria (TAB)

The average contamination of sausages with TAB is 7.13 log CFU/g, with a minimum value of 4.99 log CFU/g recorded in a supermarket, and a maximum value of 8.92 log CFU/g recorded in a butcher shop. The compliance study classifies 103/156 (66.03%) samples as unfit for consumption, 53/156 (33.97%) have an acceptable hygienic quality and 18/156 (11.54%) have a satisfactory hygienic quality. These results are higher than those found in Brazil (Santa

Table 2. Compliance percentages, minimum, maximum, average and standard deviations of microflora counted in sausages sold in Meknes city (Morocco).

	TAB	TC	FC	*E. coli*	St	Cp
The average (Log CFU)	7.13	5.05	4.06	3.69	3.43	2.42
Standard Deviation (Log CFU)	0.98	1.20	1.15	1.13	0.88	1.79
Maximum	8,92	7.36	6.62	6.59	5.6	6,05
Minimum	4.99	2.6	2.08	1.74	1.47	00
Criteria (m-M)	(5.10^5-5.10^6)	-	(10^2-10^3)	-	(5.10^2-5.10^3)	(50-(5.10^2)
Compliance percentages	33,97%	-	22,43%	-	68,59%	58,97%

TAB: Total Aerobic Bacteria, TC: Total coliforms, FC: Faecal coliforms, St: *Staphylococci*, Cp: *Clostridium perfringens, E.coli*: *Escherichia coli,* m: desired minimum threshold of contamination, M: maximum threshold of tolerable contamination.

et al., 2012) and Sudan (Abakar *et al*., 2013). Lower than those found in Turkey (Soyer *et al.*, 2005) and Spain (Banon *et al.*, 2008). Our results are comparable to those found in Greece (Ambrosiadis *et al*., 2004) and Turkey (Erkmen and Bozkurt, 2004).

Faecal coliforms

The average contamination of sausages by Faecal coliforms (CF) is 4.06 log CFU/g, it varies between a minimum of 2.08 log CFU/g recorded in a supermarket and a maximum of 6.62 log CFU/g registered among a street vendor. The compliance study has allowed us to classify 121/156 (75.34%) samples unfit for consumption, 0/156 (0%) of satisfactory quality and 35/156 (22.43%) of an acceptable quality for the consumer. These results are agreed with those found previously in Morocco (EL Allaoui *et al*., 2012).

Staphylococci

The average contamination of sausages by *Staphylococci* is 3.43 log CFU/g, the minimum and maximum values vary between 1.47 log CFU/g and 5.6 log CFU/g. These results are higher to those found in Jordan country (Mazahreh *et al*., 2013) and lower to those found in two Nigerian cities (Oluwafemi and Simisaye, 2006). However, it's similar to those found in Turkey (Siriken *et al.*, 2009). The compliance study classifies 49/156 (31.41%) samples are unfit for consumption, 32/156 (20.51%) have a satisfactory quality and 107/156 (68.59%) are acceptable for consumption.

Clostridium perfringens

The average contamination of sausages by *Clostridium perfringens* is 2.42 log CFU/g, with a minimum value of 0 CFU/g registered in several outlets and a maximum value of 6.05 log CFU/g registered in a butcher shop. These results are lower than those found previously in Morocco (EL Allaoui *et al*., 2012). A study in Casablanca (Morocco) on fermentation sausage showed the absence of these bacteria in all samples analyzed (Malti and Amarouch, 2008). The compliance study classifies 64/156 (41.03%) samples are unfit for consumption, 50/156 (32.05%) have a satisfactory quality and 92/156 (58.97%) are acceptable for consumption.

Isolation of Salmonella

Salmonella was detected in 21.79% of samples (34/156); this result corresponds to that found in Brazil (25%) (Dias *et al*., 2013) and Botswana (26%) (Mrema *et al*., 2006). Lower than that found in Algeria (46.77%) (Mezali and Hamdi, 2012) and superior to that found in Morocco (0.097%) (Bouchrif *et al.*, 2009). However, the large presence of *Salmonella* is justified by the high presence of these bacteria in the raw material (Karraouan *et al*., 2010; EL Allaoui *et al*., 2014) and the poor hygiene all along the manufacturing chain of sausages (EL Allaoui *et al*., 2012).

Season Effect on the hygienic quality of sausages

The study of seasonal effect on the contamination of sausages by total aerobic bacteria (TAB) and faecal coliforms (FC), shows that among 53/156 samples that meet the standards for TAB, 16/78 are detected in summer and 37/78 in winter with a compliance percentage of 20.51% and 47.43% respectively and among 35/156 samples that meet the standards for FC, 16/78 are detected in summer and 19/78 in winter with a compliance percentage of 20.51% and 24.36% respectively (Figure 1). The statistical analysis shows

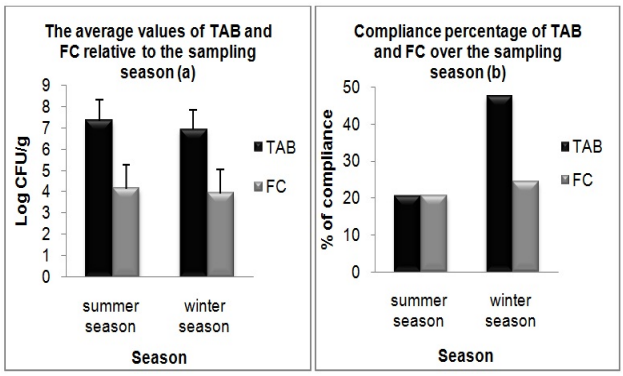

Figure 1. The average values in log CFU/g (a) and compliance percentage (b) of microflora counted (TAB and FC) in sausages according to season sampling. TAB: Total Aerobic Bacteria, FC: Faecal coliforms

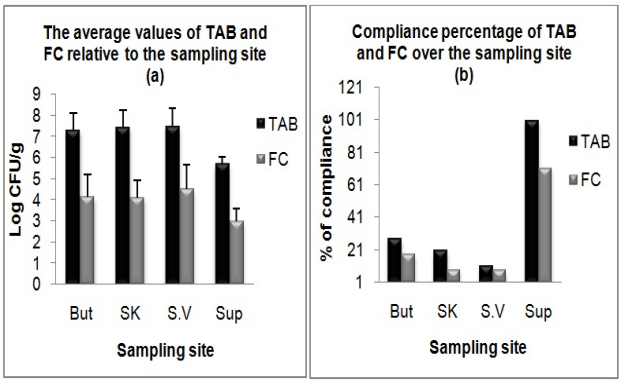

Figure 2. The averages values in log CFU/g (a) and compliance percentage (b) of microflora enumerated in sausages (TAB and FC) relative to the sampling site. TAB: Total Aerobic Bacteria, FC: Faecal coliforms, But: Butchery, SK: Souk, S.V: Street Vendors, Sup: Supermarket

that the season exerts a highly significant effect on the contamination by TAB with p = 0.00054, contrariwise the contamination with faecal coliforms remains independent of this factor (p=0.26). The increase of temperature during the summer season promotes the food contamination, increasing the percentage of non conformity during this season (Cohen *et al*., 2007; Fonkem *et al*., 2010; Marnissi *et al*., 2012).

Sampling site effect on the hygienic quality of sausages

Among the 53 samples conform to the standards hygiene according to the contamination with total aerobic bacteria (TAB), 20/72 are taken from the butchers, 4 /36 from street vendors, 5 /24 from the Souk and 24/24 from the supermarket, with a compliance percentage of 27.77%, 11.11%, 20.83% and 100% respectively (Figure 2) and among the 35 samples conform to the standards hygiene according to the contamination with faecal coliforms (FC), 13/72 are taken from the butchers, 3/36 from street vendors, 2/24 from the Souk and 17/24 from the supermarket, with a compliance percentage of

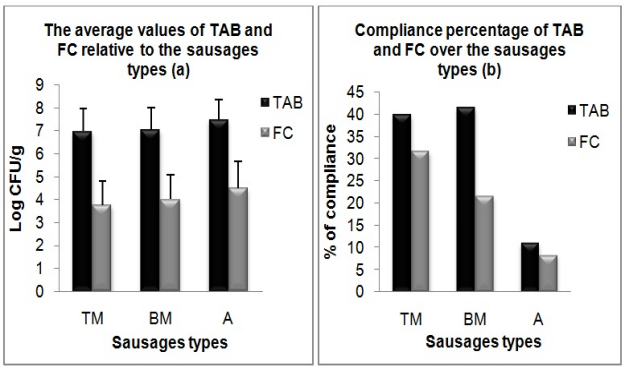

Figure 3. The average values in log CFU/g (a) and compliance percentage (b) of microflora counted (TAB and FC) relative to the sausages types. TAB: Total Aerobic Bacteria, FC: Faecal coliforms, TM: Turkey Meat, BM: Beef Meat, A: Artisanal sausages

18.05%, 8.33%, 8.33% and 70.83% respectively (Figure 2). The statistical analysis shows that there is no difference between the sausages taken from butcheries and those from the Souk (p=0.4; p=0.94) and street vendors (p=0.25; p=0.089), also between the sausages taken from the souk and street vendors (p=0.87; 0.14) for the contamination by TAB and FC respectively. However, the presence of a highly significant difference between the sausages taken from the supermarket and all other sampling sites studied with a threshold significance of P<0.001 for TAB and FC. The difference of hygienic quality between the samples taken from various sites previously cited is due to the lack of proper control during the manufacturing chain and the exposure of food products to dust and flies, especially in the open spaces, such as, the butcher, the street vendors and the souk (Ekanem *et al*., 1985; El Allaoui *et al*., 2012).

Effect of sausage type on hygienic quality

Among the 53 samples conform to the standards hygiene according to the contamination with total aerobic bacteria (TAB), we found 24/60 from turkey meat (TM), 25/60 from beef meat (BM) and 4/36 from artisanal type (A), with a compliance percentage of 40%, 41.66% and 11.11% respectively (Figure 3). As well as among 35 samples conform to the standards of hygiene according to the contamination with faecal coliforms (FC), we found 19/35 from turkey meat (TM), 13/35 from beef meat (BM) and 3/35 from artisanal type (A), with a compliance percentage of 31.66%, 21.66% and 8.33% respectively (Figure 3). The statistical analysis shows that the artisanal sausages differ significantly from the sausage of beef meat (p=0,019; p=0.04) and turkey meat (p=0.01; p=0.002) for the contamination by TAB and FC respectively. However, there is no significant difference between the sausages of beef

meat and turkey meat for the contamination by TAB (p= 0.75) and FC (p= 0.18). The undetermined origin of artisanal sausages, the traditional method of preparation, the non-respect of the good hygiene practices, and the rupture of the cold chain promote her contamination and increase the percentage of non-compliance. According to Rane (2011) the major sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse of cooked foods and the personal hygiene of vendors.

Conclusion

The result of bacteriological analysis classifies 80.77% of sausages sold in Meknes city (Morocco) do not meet the microbiological standards, for one or several criteria studied. The contamination level depends on sausage type, sampling site, and the seasonal variation intimately related to the increase of temperature. The high contamination of sausages sold in Meknes city, essentially those of artisanal sausages type sold in street vendors, reflect the non-respect of the hygiene practices throughout the manufacturing chain, storage, transport, and distribution to the various outlets. Indeed, the hygienic quality of this product can be improved through awareness programs of hygiene rules for the vendors, to respect the cold chain and to fight against food borne poisoning.

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